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Metabolism and tissue distribution of tobacco-specific N-nitrosamines in the marmoset monkey (*Callithrix jacchus*)

Andre Castonguay[1,2], Hans Tjälve[3], Neil Trushin[1], Roland d'Argy[4] and Göran Sperber[5]

[1]Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY 10595, USA, [3]Department of Pharmacology and Toxicology, Swedish University of Agricultural Sciences, Box 573, S-75123 Uppsala, [4]Department of Toxicology, and [5]Department of Physiology, University of Uppsala, S-75123, Uppsala, Sweden

[2]To whom reprint requests should be sent at School of Pharmacy, Laval University, Quebec City, P.Q. Canada G1K 7P4

Three male marmoset monkeys (*Callithrix jacchus*) were injected i.v. with the tobacco-specific carcinogen [2′-^{14}C]N′-nitrosonornicotine (NNN) (20.3 μmol/kg body weight) or [carbonyl-^{14}C]4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (18.8 or 420 μmol/kg body weight). They were sacrificed 4 h later. Tissue distribution was studied in two monkeys by whole-body autoradiography and by computer-assisted densitometric analysis of the autoradiograms. The autoradiograms showed a high level of radioactivity in the liver, nasal mucosa, kidneys, melanin of the eyes, hair-follicles of the skin and in the ceruminous ear glands of the monkeys. Total level of radioactivity was 5.7 times higher in the liver of the [carbonyl-^{14}C]NNK-injected monkey than in that of [2′-^{14}C]-NNN-injected monkey. Washing the sections with trichloroacetic acid and organic solvents selectively removed free metabolites leaving metabolites bound to cellular macromolecules. Level of bound metabolites was 1.5 times higher in the nasal mucosa than in the liver of the [2′-^{14}C]NNN monkey. Levels of bound metabolites were similar in the liver of NNN- and NNK-treated monkeys. The results indicate that the liver and nasal mucosa of *C. jacchus* can activate NNN and NNK to alkylating species. Unbound metabolites present in the liver, lung, kidneys, eye, blood and urine were extracted and separated by h.p.l.c. Hydroxylation of the carbons α to the N-nitroso group of NNN were the major metabolic pathways. Unmetabolized NNN was the major radioactive component in the liver, lung, eye and blood. Reduction of the carbonyl of NNK yields 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl). NNAl was present in all tissues analyzed and was the major radioactive component in the eye and stomach lumen. It was also excreted in the urine. NNK and NNAl were metabolized by α-carbon hydroxylation. These results suggest that in *C. jacchus*, NNN, NNK and NNAl are activated to alkylating species by α-carbon hydroxylation. In the third monkey injected with NNK, DNA methylation was observed in the liver and nasal mucosa but not in the lung and kidneys.

Pulmonary tissues of *C. jacchus*, unlike those of F344 rats, do not have the enzymic capacities to activate NNK to methylating species.

Introduction

The levels of N′-nitrosonornicotine (NNN)* and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) present in the smoke of commercial American cigarettes range from 0.1 to 0.3 μg/cigarette (1). These two tobacco-specific N-nitrosamines are also abundant in snuff tobacco manufactured in various countries (2). They induce benign and malignant tumors in A/J mice, F344 rats and Syrian golden hamsters. Their route of administration and the species of rodent used determine their organotropy (3–5). As with most N-nitrosamines, enzyme-mediated α-carbon hydroxylation has been suggested to be the initial and crucial step in the activation of NNN and NNK to electrophilic and DNA-damaging species (6–8).

Because of its small size and its facility to breed in captivity, the cotton-eared marmoset (*Callithrix jacchus*) is considered an excellent model for biomedical research. One species of marmoset, *Saquinus oedipomidas*, has been shown to develop tumors after exposure to aflatoxin B_1 (9). However, carcinogenicity or metabolism studies of N-nitrosamines have never been performed in marmoset monkeys. Considering the ubiquity of NNK in the human environment and its high carcinogenic potency in rodents, we have extended our metabolism studies to one species of primate.

We have studied the metabolism and tissue-distribution of NNN and NNK in *C. jacchus*. The results are compared to those obtained previously with three species of rodents (3–5). Levels of DNA methylation were measured in various tissues of NNK-treated monkeys and they were compared to the levels observed previously in F344 rats.

Materials and methods

Chemicals

[2′-^{14}C]NNN was obtained from New England Nuclear, Boston, MA and added to unlabeled NNN to a final specific activity of 4.2 mCi/mmol. [Carbonyl-^{14}C]NNN (sp. act.: 4.2 mCi/mmol) was prepared from [carbonyl-^{14}C]-nicotinic acid (California Bionuclear Corp., Sun Valley, CA). The radiochemical purity was >99%, as determined by h.p.l.c. (10). The syntheses of NNN and NNK and their metabolites used as reference compounds in the h.p.l.c. analyses has been reported (8,11).

Animal treatments

The three monkeys were male marmoset (*C. jacchus*) and were born in the colony of the University of Uppsala. The original breeders were imported from South America in 1976. They were fed cereals, fresh fruit and commercial pellets and were given tap water *ad libitum*. They were anesthetized by i.m. injection of ketamine chloride (Parke-Davis, Morris Plains, NJ) (10 mg/kg body weight).

The first monkey (298 g) was injectd i.v. (vena saphena) with [2′-^{14}C]NNN (25.2 μCi, 20.3 μmol/kg body weight). The second monkey (250 g) was injected i.v. with [carbonyl-^{14}C]NNK (22.7 μCi, 18.8 μmol/kg body weight) and a third monkey (298 g) was injected with [carbonyl-^{14}C]NNK (5.34 μCi, 420 μmol/kg body weight). The monkeys were sacrificed by CO_2 asphyxiation 4 h later. In

*Abbreviations: NNN, N′-nitrosonornicotine; NNN-1-N-oxide, N′-nitrosonornicotine-1-N-oxide; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAl, 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol; NNK-N-oxide, 4-(methylnitrosamino)-1-(3-pyridyl-N-oxide)-1-butanone, NNAl-N-oxide, 4-(methylnitrosamino)-1-(3-pyridyl-N-oxide)butan-1-ol; Gua, guanine; O^6-MeGua, O^6-methylguanine; 7-MeGua, 7-methylguanine.

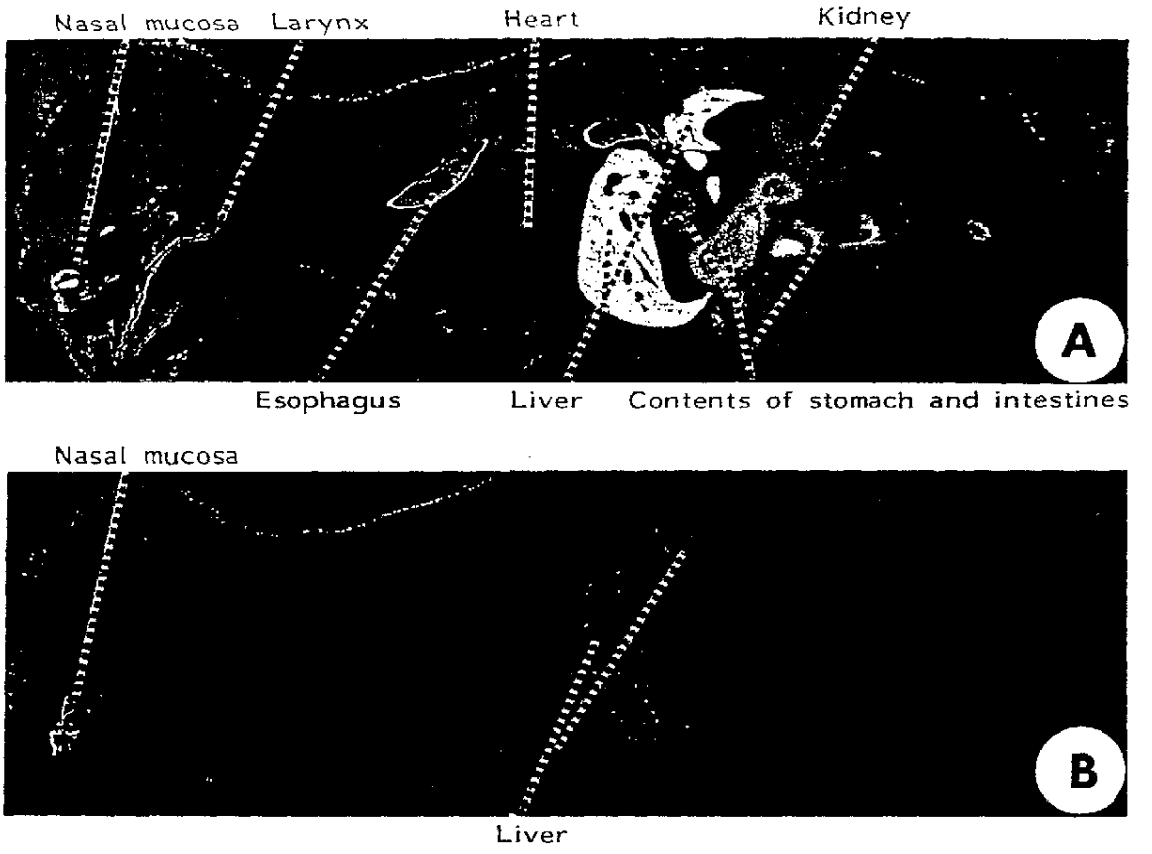

Fig. 1. Whole-body autoradiograms of marmoset monkeys 4 h after i.v. injection of [2'-^{14}C]NNN (25.2 μCi, 3.6 mg/kg). **Panel A** is an autoradiogram of a freeze-dried non-extracted section. **Panel B** is an autoradiogram of a section adjacent to A which was extracted with trichloroacetic acid and organic solvents before the autoradiographic exposure. The time of exposure was 5 months. White areas correspond to radioactivity.

rats, a significant amount of NNN and NNK was metabolized during a 4 h period [12].

Whole-body autoradiography

After sacrifice, the first two monkeys were immediately embedded in a carboxymethyl cellulose gel and sectioned sagittally on tape (20 μm thick sections) according to published procedures [13]. Twenty-five duplicate sections were taken from half of each block and were freeze-dried. The sectioning of the animals was performed on the left half of the bodies and the other half was used in the metabolism study. To localize tissue-bound metabolites, every other freeze-dried section was washed successively with 5% trichloroacetic acid, water, methanol and heptane for 0.5 min, respectively. The sections were dried and exposed to X-ray film together with the adjacent non-extracted freeze-dried sections.

Quantification of total radioactive compounds and compounds bound to cellular macromolecules by computer-assisted densitometric analysis

The densitometric system is built around a Cromemco System 2-HDG microcomputer equipped with the MC 68000 processor, 768 Kbytes of main memory, 20 Mbytes of disk memory and SDI graphics system. A simple black-and-white video camera (Philips LDH 400/61) connected to the computer through a Cromemco SDD video digitizing interface, which digitizes light intensity into 256 levels, was used to measure light, first without and then with the autoradiogram in the picture field. From these measurements, the density of the film was calculated in 241 × 369 picture elements (pixels) ~ 100 μm in size. The uniformity of the system was tested and calibrated with a set of known densities (Kodak wratten gelatin filters). The resulting picture was displayed on a RGB monitor (Barco CD233), and with the aid of a digitizer pad regions of interest were delineated for calculation of average density.

The optical densities of various tissues in the whole body autoradiograms from the [2'-^{14}C]NNN injected monkey and the [carbonyl-^{14}C]NNK-injected monkey were determined using the system described above. From the tape-fastened sections which had been used to obtain the analyzed autoradiograms, round pieces, 3 mm in diameter, were then punched out by means of a pair of tongs. The tissue pieces were dissolved in Soluene 350® and the radioactivity was determined by liquid scintillation counting. In this way, the density as well as the radioactivity levels in the liver, kidney, brain and blood could be determined. Standard curves were prepared by plotting the densities of these tissues against the corresponding liquid scintillation countings. The optical densities of the nasal mucosa, the eye melanin and the ceruminous ear glands were then determined and the radioactivity contents were obtained from the standard curves. To be able to express the values for the liquid scintillation countings on a dry weight basis, additional areas of the sections were punched out and weighed on a microbalance. By subtracting the weight of areas of tape without tissue, which were also punched, from the weight of areas with tissue plus tape, the weight of the tissues could be determined. Very small variations were found between the different punched tissues (the mean weight being 70 μg for a round area with 3 mm diameter), from a 20 μm thick section.

Quantification of total radioactive metabolites by liquid scintillation counting

Pieces of tissues obtained from the first two monkeys and weighing 10 – 150 mg were dissolved in 1 ml of Soluene 350® and the radioactivity was determined as described previously [14].

Separation and quantification of unbound metabolites

Pieces of tissues (50 – 600 mg) obtained from the first two monkeys were homogenized in 1 ml of 0.1 M HCl. After centrifugation, the pellets were dispersed in 1 ml of 0.1 M HCl. The centrifugation was repeated, the supernatants were combined and the pH was adjusted to 7.0 with 10 M NaOH. An aliquot of blood (0.5 g) was added to 2 ml of methanol. The bile (250 g) was added to 3 ml of methanol. The precipitates were washed twice with 1 ml of methanol. The combined supernatants were added to 1 ml of water and methanol was evaporated under nitrogen. The recovery of radioactivity ranged from 70 to 90% as previously shown [12]. Aliquots (100 μl) of urine were analyzed without extraction. The metabolites were separated by reverse phase h.p.l.c. and quantified by liquid scin-

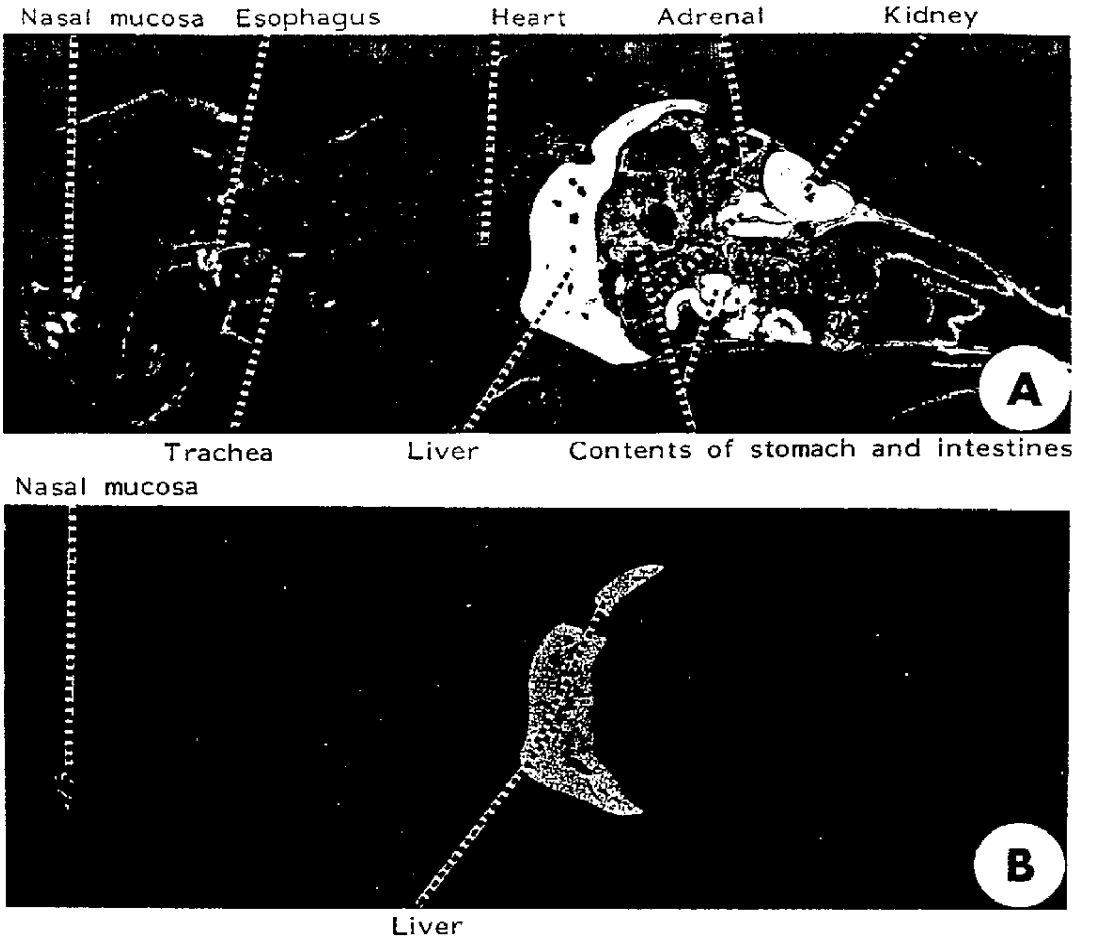

Fig. 2. Whole-body autoradiograms of a marmoset monkey 4 h after i.v. injection of [carbonyl-^{14}C]NNK (22.7 μCi, 3.9 mg/kg). **Panel A** is an autoradiogram of a freeze-dried non-extracted tissue section. **Panel B** is an autoradiogram of a tissue section adjacent to **A**, which was extracted with trichloroacetic acid and organic solvents before the autoradiographic exposure. The time of exposure was 2 months.

filtration as described previously (5,11). The amount of nasal tissue available was too small to allow the identification of the NNN and NNK metabolites present in this tissue.

Comparison of the glycine conjugates of 10 and 12 with the major NNK metabolite present in the liver

A polar metabolite eluting at 11 ml accounted for 79% of the radioactive metabolite present in the liver but did not co-elute with any of NNK metabolites observed previously in rodents (3,5,8). Putative metabolites were synthesized and their retention volume was compared with that of the unknown metabolite. Glycine conjugates of the keto acid 10 and hydroxy acid 12 were synthesized as follows. Glycine methyl ester was reacted with 10 using dicyclohexyl carbodiimide as dehydrating agent. The ester was hydrolyzed to the carboxylic group with diluted NaOH. The glycine conjugate of the keto acid 10 was also treated successively with sodium borohydride and diluted NaOH to give the glycine conjugate of the hydroxy acid 12. Neither of the two glycine conjugates co-elute with the unknown NNK metabolite.

Assay of methylated Gua

DNA was extracted from tissues of the third monkey injected with NNK (420 μmol/kg body weight). As described by Daoud and Irving (15), this extraction procedure includes the removal of RNA by digestion with pancreatic ribonuclease A (E.C. 3.1.27.5) for 30 min at room temperature. Guanine (Gua) and methylated Gua were separated by cation exchange h.p.l.c. (16). DNA samples (0.3 – 10.0 mg) were dissolved in 10 mM sodium cacodylate (1 mg/0.2 ml, pH 7.0) and incubated at 100°C for 30 min. HCl (1 M) was added to the ice-cold sample. Final concentration of HCl was 0.1 M. After centrifugation, 100 μl of the supernatant was injected on the column to measure the levels of 7-MeGua. The pellet was dissolved in 0.1 M HCl (1 mg/0.2 ml) and incubated at 80°C for 30 min. Gua and O^6-MeGua were separated by h.p.l.c. and their levels measured by u.v. spectrophotometry and fluorimetry, respectively.

Results

Whole-body autoradiography

The autoradiograms obtained from the [2′-^{14}C]NNN-injected monkey showed the highest levels of radioactivity to be present in the liver, the nasal mucosa, the melanin of the eyes, the hair follicles of the skin, and the ceruminous ear glands (Figures 1A and 3A and C). Radioactivity was also present in the kidneys, and in the stomach, intestine and urinary bladder lumens. Labeling of the tracheo-bronchial and esophageal mucosa exceeded somewhat the homogeneously distributed tissue radioactivity. In all other tissues, labeling was homogeneous and about equal to that present in the blood. In autoradiograms of tissue sections washed with trichloroacetic acid and organic solvents, bound radioactive metabolites were observed in the liver and nasal mucosa (Figure 1B). The part of the tissue-labeling which was retained in the washed sections of the [2′-^{14}C]NNN-injected monkey was higher in the nasal mucosa than in the liver. The washings removed the radioactivity present in all other tissues.

The labeling of the liver was higher in the [carbonyl-^{14}C]NNK- than in the [2′-^{14}C]NNN-injected monkey (Figure 2A). Labelings of the other organs of both animals were comparable (Figures 1 – 3). Radioactive metabolites bound to cellular macromolecules were observed only in the liver and nasal mucosa (Figure 2B). In the latter tissue, the level of bound radioactivity was higher in the [2′-^{14}C]NNN- than in the [carbonyl-^{14}C]NNK-injected monkey.

Computer-assisted densitometric analysis

The levels of radioactivity present in various tissues were measured by computer-assisted densitometric analysis of whole-body autoradiograms. As shown in Table I, the level of radioactivity in the liver was 5.7 times higher in the [carbonyl-^{14}C]NNK-injected monkey than in the [2′-^{14}C]NNN-injected monkey. However levels of radioactivity present in the nasal mucosa and eye melanin were higher in the [2′-^{14}C]NNN monkey. Metabolites bound to cellular macromolecules were present in the liver and nasal mucosa of both monkeys. Metabolites bound to melanin were observed only in the [2′-^{14}C]NNN-injected monkey.

Levels of total radioactive compounds present in various tissues were also measured by scintillation counting. The levels (nmol/g of wet tissue) in the [2′-^{14}C]NNN-injected monkey were: liver, 30.9; kidney, 17.9; lung, 8.1; esophagus, 11.5; adrenal, 11.3. In the [carbonyl-^{14}C]NNK-injected monkey, the levels of radioactivity were: liver, 187.5; kidney 32.5; lung, 11.5; esophagus, 10.5; adrenal, 28.3. Using the data published by Wadsworth (17) on marmoset organ weight, the total amount of radioactive metabolites present in various organs can be calculated. The amount of radioactive metabolites present in the liver and kidneys of the [2′-^{14}C]NNN-injected monkey was equal to ~7% and 0.5% of the dose. In the [carbonyl-^{14}C]NNK-injected monkey, ~40% and 0.8% of the injected dose was present in the liver and kidneys respectively.

The metabolic pathways of NNN are illustrated in Figure 4. Pyridine-N-oxidation and denitrosation of NNN give the NNN-1-N-oxide (1) and norcotinine (2), respectively. Hydroxylation of the 2′-carbon leads to the electrophilic diazohydroxide 5 which reacts with water to ultimately give the keto acid 10 and diol 11. Hydroxylation of the 5′-carbon of NNN gives the hydroxy acid 12 as an end product. Levels of NNN and its metabolites present in various tissues are summarized in Table II. Oxidations of NNN to 1 and 2 are two minor metabolic pathways and both metabolites were present in the urine. The keto acid 10 was detected only in the liver, kidney, eyes and urine. In contrast, the hydroxy acid 12 was observed in all tissues and was 10 times more abundant that the keto acid 10 in the urine. Unmetabolized NNN was also present in all tissues and was the major radioactive component in the liver, lung, eye and blood. NNN-1-N-oxide (1), keto acid 10, and hydroxy acid 12 were also present in

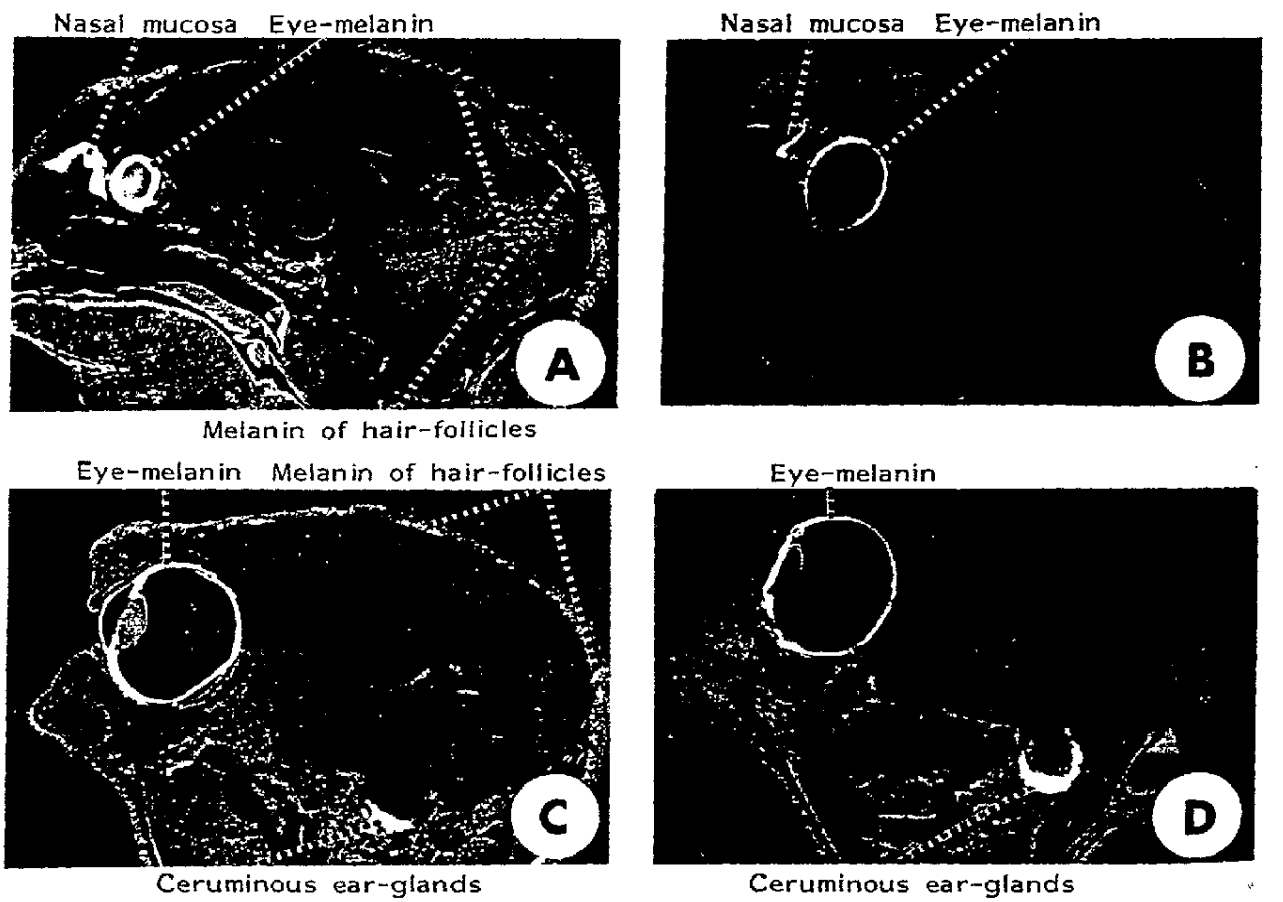

Fig. 3. Details of whole-body autoradiograms of marmoset monkeys 4 h after i.v. injection of [2′-^{14}C]NNN (25.2 μCi; 3.6 mg/kg) panels A and C or [carbonyl-^{14}C]NNK (22.7 μCi; 3.9 mg/kg body weight) panels B and D. The time of exposure was 5 months for A and C and 2 months for C and D.

the gastric lumen and were less abundant than NNN (data not shown).

As shown in Figure 5, carbonyl reduction of NNK yields NNAl. Pyridine-N-oxidation of NNK and NNAl give the N-oxides 13 and 14, respectively. While the end-products of α-carbon hydroxylation of NNK are the keto alcohol 7 and keto acid 10, similar metabolic pathways with NNAl lead to hydroxy acid 12. Levels of NNK and NNAl metabolites present in various tissues of the second monkey are summarized in Table III. NNAl was present in all tissues and was excreted in relatively large quantity in the urine. NNK was present only in small quantities, in the lungs, kidneys and eyes. In all tissues, the level of hydroxy acid 12 was higher than the keto alcohol 7 + keto acid 10. NNAl constituted ~86% of the total radioactive metabolites present in the stomach lumen. In the third monkey which was injected with a larger amount of NNK, NNAl constituted 43% of the total extractable radioactive compounds present in the liver and the ratio NNK/NNAl was 0.04.

As shown in Table IV, methylated guanines were observed in the DNA of the liver and nasal mucosa but not in the DNA of the lungs and kidneys.

Table I. Total levels of radioactive compounds and levels of radioactive compounds bound to cellular macromolecules insoluble in trichloroacetic acid in various *Callithrix jacchus* tissues

Tissues	Levels of radioactive compounds (nmol/g of dry tissue)			
	[2'-^{14}C]NNN		[Carbonyl-^{14}C]NNK	
	Total	Bound	Total	Bound
Liver	141	19	810	20
Kidney	57	N.D.[a]	191	N.D.
Brain	41	N.D.	34	N.D.
Blood	34	N.D.	78	N.D.
Nasal mucosa	187	32	159	23
Eye melanin	196	32	165	N.D.
Ceruminous ear glands	141	N.D.	236	N.D.

[a]N.D. = no detectable radioactivity.
Whole-body autoradiograms were prepared from two monkeys injected i.v. with either [2'-^{14}C]NNN (20.3 μmol/kg) or [carbonyl-^{14}C]NNK (18.8 μmol/kg body weight) and killed 4 h later. The shades of gray in the autoradiograms were analyzed in a computerized image system as described in Materials and methods.

Discussion

N-Nitrosamines have been shown to be carcinogenic in >32 animal species (18) including species of non-human primates (19). Metabolism studies of various N-nitrosamines in many of these species have suggested that hydroxylation of the carbon adjacent to the N-nitroso group (α-carbon) is the initial and crucial step

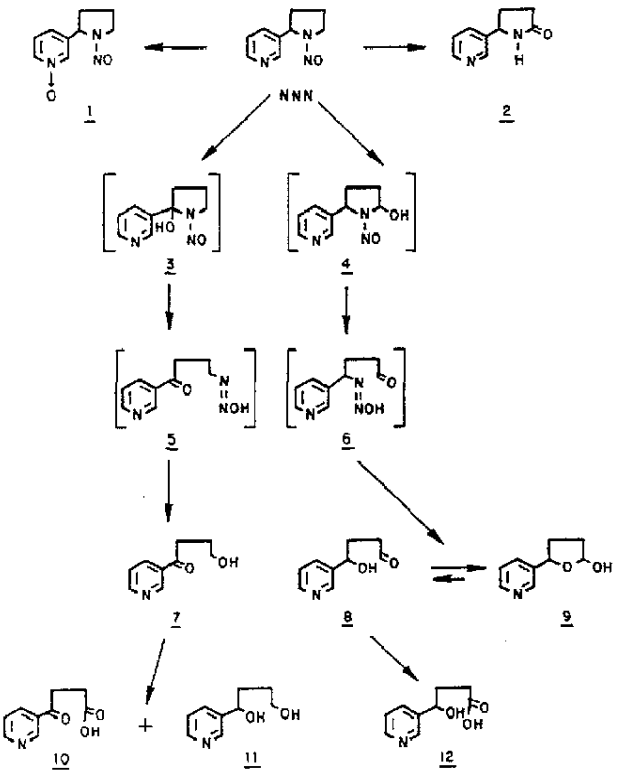

Fig. 4. Metabolic transformations of NNN in intact cell systems. Structures in brackets are hypothetical intermediates.

Table II. Levels of NNN and its metabolites in some tissues of a marmoset monkey after i.v. injection of [2'-^{14}C]NNN

Metabolites[a]	Levels of NNN and its metabolites (nmol/g of wet tissue or fluid)[b]						
	Liver	Lung	Kidney	Eye	Blood	Bile	Urine[c]
NNN	2.9	3.9	2.7	7.7	3.4	5.5	20 (0.14)
NNN-1-N-oxide 1	–[d]	–	–	0.4	–	–	62 (0.45)
Norcotinine 2	1.1	–	–	–	0.9	2.1	25 (0.18)
Keto acid 10	0.7	–	1.4	1.7	–	–	74 (0.53)
Diol 11	–	–	–	–	–	–	3.3 (0.02)
Hydroxy acid 12	0.9	0.9	3.6	0.1	0.5	4.6	830 (5.95)
Unknown[e]	3.2	–	–	–	–	6.4	13 (0.07)

One monkey was anesthetized, injected with [2'-^{14}C]NNN (25.2 μCi, 3.6 mg/kg body weight) and sacrificed 4 h later. The unbound metabolites present in various tissues were extracted and quantified by h.p.l.c. and liquid scintillation as described in Materials and methods.
[a]Numbers refer to Figure 4.
[b]Mean of values obtained from duplicate analyses.
[c]The total level of radioactive compounds in the urine excreted (430 μl) corresponded to 10% of the injected dose. Numbers in parentheses are percentages of the injected dose.
[d]– not detected. Limit of detection was 0.1 nmol/g of wet tissue corresponding to twice the baseline radioactivity.
[e]Retention volume was 16–22 ml.

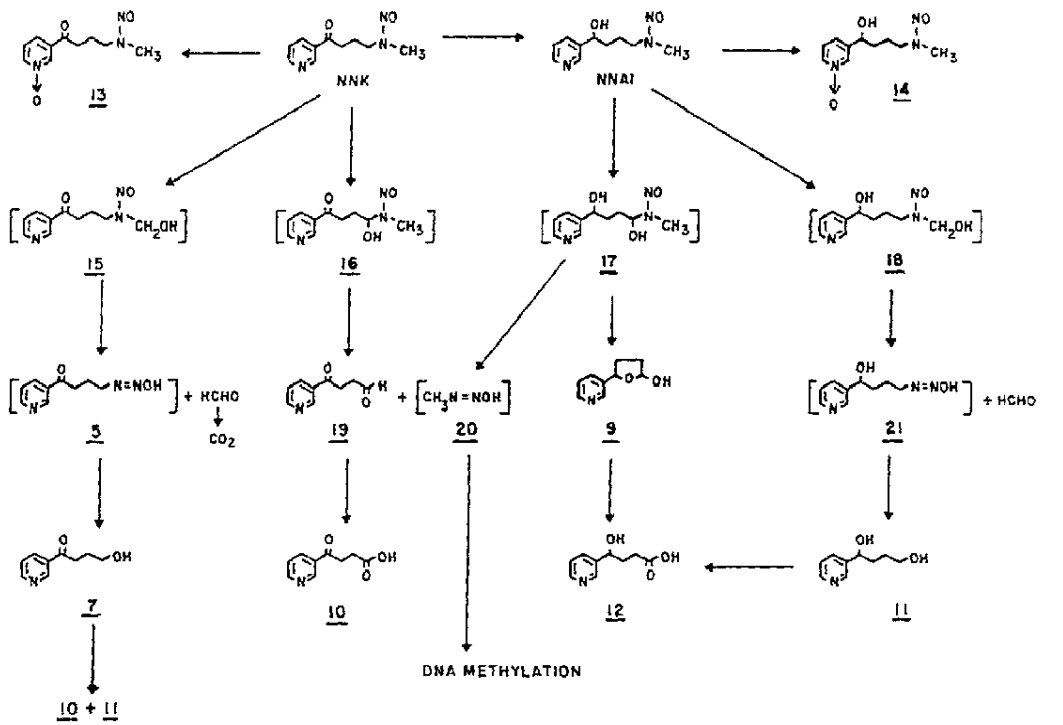

Fig. 5. Metabolic transformations of NNK and NNAl in intact cell systems. Structures in brackets are hypothetical intermediates.

Table III. Levels of NNK, NNAl and their metabolites in some tissues of a marmoset monkey 4 h after i.v. injection of [carbonyl-^{14}C]NNK

Metabolites[a]	Levels of NNK, NNAl and their metabolites (nmol/g of wet tissue or fluid)[b]					
	Liver	Lung	Kidney	Eye	Blood	Urine[c]
NNK	–[d]	0.1	0.2	0.2	–	–
NNAl	0.8	4.3	5.3	10.9	3.3	136 (1.0)
Keto alcohol 7	–	–	–	–	–	17.6 (0.13)
Keto acid 10	1.5	0.4	0.4	–	0.1	37.9 (0.28)
Hydroxy acid 12	12.9	3.6	13.7	–	2.7	297.1 (2.20)
NNK-N-oxide 13	9.2	0.1	0.3	–	–	–
NNAl-N-oxide 14	–	0.5	–	–	0.1	38.8 (0.29)
Unknown[e]	91	–	–	–	–	–

One monkey was anesthetized, injected with [carbonyl-^{14}C]NNK (22.7 μCi, 3.9 mg/kg body weight) and sacrificed 4 h later. The unbound metabolites present in various tissues were extracted and quantified by h.p.l.c. and liquid scintillation as described in Materials and methods.

[a]Numbers refer to Figures 4 and 5.

[b]Mean of values obtained from duplicate analyses.

[c]The total level of radioactive compounds in the urine excreted (400 μl) corresponded to 4.5% of the injected dose. Numbers in parentheses are percentages of the injected dose.

[d]– not detected. Limit of detection was 0.1 nmol/g of wet tissue corresponding to twice the baseline radioactivity.

[e]Retention volume was 8–18 ml.

in their bioactivation (6). The results of the present study indicate that *C. jacchus* is also a species which can α-carbon hydroxylate N-nitrosamines. The most abundant metabolites present in various tissues or excreted in the urine were formed by this pathway.

Previous autoradiographic studies with C57BL mice, F344 rats or Syrian golden hamsters have shown that the major site of activation of NNN and NNK to alkylating species was the liver (12,14,20,21). This observation also applied to *C. jacchus*.

The nasal mucosae of rats and hamsters are particularly sensitive to the carcinogenic properties of NNN and NNK (4,5). This was associated with a relatively high labeling of the nasal mucosae in autoradiograms of these rodents treated with ^{14}C-labeled NNN or NNK (12,14,21). A marked labeling of the nasal mucosae was also observed in *C. jacchus* and the autoradiograms suggest that the nasal mucosae was especially effective in activating NNN. The carcinogenic potency of N-nitrosamines in this species of monkey has not been determined. However previous studies have shown that N-nitrosodiethylamine is a strong nasal carcinogen in the bush baby (*Galago crassicaudatus*)

Table IV. Levels of O^6-MeGua and 7-MeGua in DNA of some tissues of marmoset monkey 4 h after i.v. injection of [carbonyl-^{14}C]NNK

Tissues	O^6-MeGua/Gua (μmol/mol)	7-MeGua/Gua (μmol/mol)
Liver	59[a]	636[a]
Nasal mucosa	16[b]	106[b]
Lungs	N.D.[c]	N.D.
Kidneys	N.D.	N.D.

One monkey (298 g) was anesthesized, injected with NNK (420 μmol/kg body weight) and sacrificed 4 h later. DNA was extracted from four tissues and hydrolyzed. Gua, O^6-MeGua and 7-MeGua were measured by h.p.l.c.-fluorimetry as described in Materials and methods.

[a]Mean of two determinations.

[b]Single determination.

[c]N.D. not detected. Limit of detection for O^6-MeGua was 0.6 pmol corresponding to 3 μmol/mol Gua when 0.8 μmol DNA nucleobases were analyzed. Limit of detection for 7-MeGua was 6 pmol corresponding to 30 μmol/mol Gua when 0.8 μmol of DNA nucleobases were analyzed.

(19). The higher labeling of the nasal mucosa in the monkeys injected with [2'-^{14}C]NNN than in the one treated with [carbonyl-^{14}C]NNK could be due to a less efficient hepatic metabolism of NNN. This would result in a comparatively higher accumulation of NNK in the nasal mucosa.

The uveal and retinal melanin of the eye and the melanin of the hair follicles of *C. jacchus*, like those tissues of C57Bl mice and Syrian golden hamsters were strongly labeled after the administration of ^{14}C-NNN or ^{14}C-NNK. Melanin has the structure of a polyanion and binds electrostatically basic compounds like NNN and NNK (22). In the NNK-injected monkey, a significant part of radioactivity was due to NNAl. This probably reflects a rapid reduction of NNK to NNAl in the liver, resulting in a high level of this metabolite in the blood. The NNAl would accumulate in melanin-containing cells by binding electrostatically to melanin.

The urinary tract and lower digestive tract of rodents and *C. jacchus* play similar roles in the excretion of NNN and NNK metabolites. NNN and NNAl were present in the gastric contents of those animals due to protonation and trapping in the gastric juice (12,20,21). They were eliminated into the duodenum. In rodents, only a small fraction of the radioactive dose was excreted in the feces suggesting that NNN and NNK and their metabolites are absorbed from the intestine. The labeling of the kidneys and lumen of the urinary bladder of the *C. jacchus* like those of rodents suggests that they are involved in the excretion of NNN and NNK metabolites. In *C. jacchus* as in F344 rats, the hydroxy acid 12 was a urinary metabolite more abundant than the keto acid 10 (8).

In previous studies (23,24) F344 had been treated i.v. with NNK (0.42 nmol/kg body weight) and sacrificed 4 h later as with the third monkey used in the present study. Levels of O^6-MeGua/Gua (470 μmol/mol) and 7-MeGua/Gua (470 μmol/mol) in rat liver were similar to those observed with marmoset monkeys. This suggests that the liver of these two phylogenetically different species have similar capacities to activate NNK to methylating species. Labeling of the liver as shown in Figure 2B and the methylation of hepatic DNA (Table IV) indicate that methyl hydroxylation and α-methylene hydroxylation are both important activation pathways in marmoset liver (see Figure 5). Interestingly, the level of O^6-MeGua/Gua in the rat nasal mucosa (219 μmol/mol) (22) was 13 times higher than marmoset nasal mucosa. Thus, the nasal mucosa of the monkey is clearly less effective in activating NNK to alkylating species. This is further demonstrated by comparing the ratio of macromolecule oxobutylation by [carbonyl-^{14}C]NNK in the nasal mucosae and in the liver. This ratio is 5.8 in F344 rats (24) but only 1.1 in marmoset monkeys (Table I).

NNK was found to be a potent lung carcinogen in rats (25) and the level of O^6-MeGua/Gua in the lung of NNK-treated rats was 13 μmol/mol (24). In whole-body autoradiographic studies, labeling of rat lung by [carbonyl-^{14}C]NNK was limited to the bronchial mucosae (12). In the lung of marmoset monkeys, no DNA methylation was observed. The labeling of the bronchial mucosae was removed by acid washing, indicating that no oxobutylation had taken place. In spite of the fact that all species treated with NNK so far (F344 rats, A/J mice and Syrian golden hamsters), have been shown to develop lung carcinomas, the results of the present study suggest that the lungs of some animal species might be less susceptible to the carcinogenic properties of NNK.

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